

Reports
Screw worm
APR 22 1957

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH ~~BRANCH~~ DIVISION

P. O. Box 3391
Orlando, Florida
February 18, 1957

AIRMAIL

To: R. C. Bushland, Box 232, Kerrville, Texas
From: C. L. Smith
Subject: Special Report--Screw-worm Activities

In response to Mr. Graham's request in his memorandum of April 1, on this subject, we have enclosed the original and one copy of the screw-worm activities of the Orlando, Florida sublaboratory. The report covers the period October 1, 1956 to April 30, 1957. The Clewiston field test results have been omitted since these results were presented in the Annual Progress Report.

Enc. (2)

C. L. Smith

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGICAL RESEARCH DIVISION

R. O. Fox 3391
Orlando, Florida
February 10, 1951

AIRMAIL

To: R. C. Sealander, Box 934, Fort Worth, Texas

From: G. L. Smith

Subject: Social Report--Green-worm activities

In response to Mr. Sealander's request in his memorandum of April 1, on this subject, we have enclosed the original and one copy of the green-worm activities of the Orlando, Florida substation. The report covers the period October 1, 1950 to April 30, 1951. The classification of this report have been checked and these results were presented in the Annual Progress Report.

Enc. (2)

Summary

Special Report--Screw-worm Activities
Orlando, Florida, April 30, 1957

Work Project ENT h2 Flies affecting man and livestock, Investigations of
Research Line Project ENT h2-15 Development of improved media and mass
rearing and distribution techniques for screw-worm eradication.

Results of preliminary tests with whale meat substituted for either ground beef, horse meat or beef heart in the regular rearing medium indicate that larvae can be satisfactorily grown on it. Adjustment of the regular formula is indicated for best results as the larvae produced were slightly inferior to those produced on the regular formula.

Preliminary tests indicate a direct relationship in both survival and mating between quantity of food provided and quality of insects.

Tests indicate that polyethylene sheets can be satisfactorily used as liners for the rearing vats to reduce the cost of cleaning the vats. Cost per sheet is estimated to be about 35-45 cents.

Information obtained in a few tests indicate that method of egging flies influences the quality of larvae produced.

The old methods of egging flies has been discarded in favor of using pint jars in which the attractant is placed.

A large cage designed to hold as many flies as 60 standard colony cages proved successful in preliminary tests though some refinements are indicated for completely satisfactory results.

Summary

Special Report--Screw-worm Activities
Orlando, Florida, April 30, 1957

Work Project RMT HS Flies affecting man and livestock, Investigations of
Research Line Project RMT HS-15 Development of improved media and mass
rearing and distribution techniques for screw-worm eradication.

Results of preliminary tests with whole meat substituted for either
ground beef, horse meat or beef heart in the regular rearing medium indicate
that larvae can be satisfactorily grown on it. Adjustment of the regular
formula is indicated for best results as the larvae produced were slightly
inferior to those produced on the regular formula.

Preliminary tests indicate a direct relationship in both survival
and mating between quantity of food provided and quality of insects.

Tests indicate that polyethylene sheets can be satisfactorily used
as liners for the rearing vats to reduce the cost of cleaning the vats. Cost
per sheet is estimated to be about 35-45 cents.

Information obtained in a few tests indicate that method of egg-
ing influences the quality of larvae produced.

The old methods of egg-ing flies has been discarded in favor of
using pint jars in which the attractant is placed.

A large cage designed to hold as many flies as 60 standard colony
cages proved successful in preliminary tests though some refinements are
indicated for completely satisfactory results.

Results of tests in which screw-worm pupae were submerged in varying degrees of wet sand indicate a delay in emergence approximately equal to the time submerged.

A tabulation of results of the ovipositional response of fertile-mated screw-worm female flies compared to that of sterile-mated females is presented.

The influence of various adult screw-worm feeding methods on oviposition as induced by attractants and vials methods is shown in tabular form. Honey-meat mixture plus honey alone the entire period did not show an advantage in eggs produced or pupal recovery over the standard of the honey-meat being replaced after the third day with honey alone.

Results of tests using irradiated female screw-worm flies in relation to age after treatment with notes on normal female longevity are given.

Various types of containers for fly releases were developed and preliminary tests with these are discussed.

Collections from status traps in North Florida indicate screw-worms to be moderately active throughout the state, except the extreme western part. Screw-worms were also active in southern Georgia as late as February. Flies were also still active in the Clewiston test area as determined from catches from traps.

Results of tests in which screw-worm pupae were submerged in varying

degrees of wet sand indicate a delay in emergence approximately equal to the

time submerged.

A tabulation of results of the ovipositional response of fertile-

mated screw-worm female flies compared to that of sterile-mated females is

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Results of tests using irradiated female screw-worm flies in

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Collections from status traps in North Florida indicate screw-

worms to be moderately active throughout the state, except the extreme

western part. Screw-worms were also active in southern Georgia as late as

February. Flies were also still active in the Gwinston test area as

determined from catches from traps.

Research Line Project ENT h2-16 Development of attractants for estimating and controlling natural screw-worm populations

Preliminary tests indicated that juices from thawed ground beef, beef heart, and ground horse meat were attractive to screw-worm flies when they had been incubated for 36-48 hours. Also attractive to varying degrees were 15 grams dried blood albumin, 5 grams animal fat, 300 cc. distilled water; 15 grams dried blood albumin and 300 cc. distilled water; 4 grams powdered milk in 400 cc. hemolized blood, all incubated for varying intervals.

Further tests with the above materials have led to a standardized testing procedure in the laboratory which gives rather consistent results. Results of these tests are given and the methods of testing are discussed.

Research Line Project ENT 12-16 Development of attractants for estimating and controlling natural screw-worm populations

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Further tests with the above materials have led to a standardized testing procedure in the laboratory which gives rather constant results. Results of these tests are given and the methods of testing are discussed.

Special Report--Screw-worm Activities
Orlando, Florida, April 30, 1957

Work Project ENT h2 Flies affecting man and livestock, Investigations of

Research Line Project ENT h2-15 Development of improved media and mass rearing and distribution techniques for screw-worm eradication

A. Mass rearing techniques

1. Media studies

- a. Preliminary test, whale meat vs. horse meat in the larval-rearing medium (Graham, Dudley)

Approximately 4,500 larvae were reared through on a medium containing whale meat. For comparison approximately 6,000 larvae were reared through on a medium containing horse meat. Each medium was infested with equal numbers of newly hatched larvae. Standard formulations were used for both media. The consistency of the medium containing whale meat was satisfactory, but not quite as firm as the medium containing horse meat. Mature larvae grown on horse meat averaged 85 mg. while larvae grown on the medium of whale meat averaged 79 mg.

- b. Whale meat compared to horse meat in larval medium (Baumhover, New)

Recently 70 pounds of whale meat were obtained for testing as a component of the larval medium. Since horse meat of good quality was available, it was used for comparison. Four lots of 500 mg. (10,000) eggs were obtained by the vial method from the 12th generation Bithlo A strain (BA F₁₂). Two pans of each were reared through in the usual manner with the following results.

Work Project ENT H2 Flies affecting man and livestock, Investigations of

Research line Project ENT H2-15 Development of improved media and mass
rearing and distribution techniques for screw-worm eradication

A. Mass rearing techniques

1. Media studies

- a. Preliminary test, whale meat vs. horse meat in the
larval-rearing medium (Graham, Dudley)

Approximately 1,500 larvae were reared through on a medium

containing whale meat. For comparison approximately 6,000 larvae were reared

through on a medium containing horse meat. Each medium was infested with
equal numbers of newly hatched larvae. Standard formulations were used for
both media. The consistency of the medium containing whale meat was satis-
factory, but not quite as firm as the medium containing horse meat. Mature
larvae grown on horse meat averaged 85 mg. while larvae grown on the medium
of whale meat averaged 79 mg.

- b. Whale meat compared to horse meat in larval medium
(Bannhaver, New)

Recently 70 pounds of whale meat were obtained for testing

as a component of the larval medium. Since horse meat of good quality was
available, it was used for comparison. Four lots of 500 mg. (10,000) eggs
were obtained by the viral method from the 12th generation Bitilo A strain
(BA F12). Two pans of each were reared through in the usual manner with

the following results.

Pan No.	Material	: : : Liters: Pounds:		: : : cc's. : No. of :		: : : Total: : Pounds :		: : : Average : Wt. of	
		: medium:	: meat :	: pupae :	: pupae :	: No. of:	: per 1,000:	: larvae	: in mg.
		: used :	: used :	: produced:	: per cc.:	: pupae :	: larvae :		
1	Horse meat	8.4	7.42	798	8.2	6,544	1.13	74.6	
2	do.	8.4	7.42	735	8.2	6,027	1.23	75.0	
3	Whale meat	6.4	5.65	334	11.6	3,874	1.46	54.2	
4	do.	6.4	5.65	340	11.2	3,808	1.48	54.2	

Considerable effort was made to avoid waste of medium so that an indication of the relative efficiency of the 2 materials might be obtained. Although the rate of growth of larvae in both media appeared the same during the first 48 hours, after this time rate of growth in the horse meat medium was noticeably more rapid than in the whale medium. Since medium was added at the same rate in both groups through the 3rd day, less efficiency may have been obtained from part of the whale medium due to a longer period of incubation before utilization. In addition, whale medium requirements may have been calculated on the low side due to less rapid utilization of the medium, possibly accounting in part for the smaller size of the larvae compared to those raised on horse meat.

Although horse meat medium in this test was superior in all respects to whale--size of larvae, percent larval recovery, and pounds of meat per 1,000 larvae--the results are considered sufficiently encouraging to warrant procurement of greater quantities of whale meat for further testing.

c. Effect of larval food limitation on efficiency of screw-worm production and quality of the insects produced
(Baumhover, New, Dudley)

During Dr. Fraenkel's visit (insect physiologist) one of the suggestions made to decrease the cost of screw-worm rearing was limitation of

No.	Material	used	produced	per cc. pupae	larvae in mg.	Pan	medium	most	pupae	No. of	Total	Wt. of	Pounds	Average
1	Horse meat	8.4	7.42	798	8.2	6.24	1.13	74.6						
2	do.	8.4	7.42	732	8.2	6.027	1.23	72.0						
3	Whale meat	6.4	5.62	334	11.6	3.874	1.46	24.2						
4	do.	6.4	5.62	340	11.2	3.808	1.48	24.2						

Considerable effort was made to avoid waste of medium so that an indication of the relative efficiency of the 2 materials might be obtained. Although the rate of growth of larvae in both media appeared the same during the first 48 hours, after this time rate of growth in the horse meat medium was noticeably more rapid than in the whale medium. Since medium was added at the same rate in both groups through the 3rd day, less efficiency may have been obtained from part of the whale medium due to a longer period of incubation before utilization. In addition, whale medium requirements may have been calculated on the low side due to less rapid utilization of the medium, possibly accounting in part for the smaller size of the larvae compared to those raised on horse meat.

Although horse meat medium in this test was superior in all respects to whale--size of larvae, percent larval recovery, and pounds of meat per 1,000 larvae--the results are considered sufficiently encouraging to warrant procurement of greater quantities of whale meat for further testing.

c. Effect of larval food limitation on efficiency of screw-worm production and quality of the insects produced (Barnhaver, New, Dudley)
During Dr. Fraenkel's visit (insect physiologist) one of the suggestions made to decrease the cost of screw-worm rearing was limitation of

larval food to produce smaller but more flies per pound of meat. Consequently duplicate pans of larvae were reared using 4,400, 6,400, and 8,400 cc's (control) of medium. Bithlo "A" F₁₀ flies were egged by the vial method to obtain 500 mg. or 10,000 eggs for each pan. At 54 hours all pans had received a total of 4,400 cc's of medium. No additional medium was provided for pans 1 and 2 but at 70 hours pans 3, 4, 5, and 6 each received an additional 2,000 cc's of medium and at 85 hours the final feeding of 2,000 cc's of medium was made in pans 5 and 6. Data from this test are contained in table 1.

Table 1.--Effect of larval food limitation on screw-worm production and male longevity and mating frequency.

Production						: Longevity and mating frequency										
						:Percent flies surviving:Theoretical										
:	:	:	:	:	:	: Pounds		: Indicated number		:	: average					
:	: cc's.	:Number	: Pupae	:	:	:meat per:		: of days		:	:number of					
Pan:	medium	: of	: per cc.	:	: Percent	: 1,000	:	: Males	:	: Females ^{1/}	:	: matings				
No.:	provided	: pupae	: volume	:	: emergence:	: larvae	:	: 3	:	: 8	:	: 3	:	: 8	:	:per male
1	4,400	4,755	10.3-14.8	94.2-86.6	0.82	88	68	---	98	6.8						
2	4,400	4,788	13.7-17.0	93.6-44.4	0.81	---	---	---	---	---						
3	6,400	4,583	8.9	96.8	1.23	80	74	---	96	8.7						
4	6,400	5,517	9.0	91.7	1.03	---	---	---	---	---						
5	8,400 (control)	6,833	7.7-8.1	97.4	1.08	100	94	---	95	9.6						
6	8,400 (control)	6,083	7.6-8.2	91.9	1.22	---	---	---	---	---						

^{1/} All females received nonlimited food in larval stage.

As expected, smaller and somewhat fewer larvae were produced in the pans receiving limited amounts of medium, compared to the controls (8,400 cc) in which the amount was not limited. Emergence was satisfactory for all groups

larval food to produce smaller but more flies per pound of meat. Consequently duplicate pans of larvae were reared using 4,400, 6,400, and 8,400 cc's (control) of medium. Rithmo "A" F10 flies were egged by the vital method to obtain 500 mg. or 10,000 eggs for each pan. At 24 hours all pans had received a total of 4,400 cc's of medium. No additional medium was provided for pans 1 and 2 but at 70 hours pans 3, 4, 5, and 6 each received an additional 2,000 cc's of medium and at 85 hours the final feeding of 2,000 cc's of medium was made in pans 5 and 6. Data from this test are contained in table I.

Table I.--Effect of larval food limitation on screw-worm production and male longevity and mating frequency.

Pans	No. provided	Pupae	Volume	Per cc.	Percent	Males	Females	Matings	Number of	Average	Percent flies surviving	Longevity and mating frequency
1	4,400	4,755	10.3-14.8	91.2-86.6	0.82	88	68	—	98	6.8	—	—
2	4,400	4,788	13.7-17.0	93.6-44.4	0.81	—	—	—	—	—	—	—
3	6,400	4,583	8.9	96.8	1.23	80	74	—	96	8.7	—	—
4	6,400	2,517	9.0	91.7	1.03	—	—	—	—	—	—	—
5	8,400 (control)	6,833	7.7-8.1	97.4	1.08	100	94	—	95	9.6	—	—
6	8,400 (control)	6,083	7.6-8.2	91.9	1.22	—	—	—	—	—	—	—

1/ All females received nonlimited food in larval stage.

As expected, smaller and somewhat fewer larvae were produced in the pans receiving limited amounts of medium, compared to the controls (8,400 cc) in which the amount was not limited. Emergence was satisfactory for all groups

except the final pupal separation from pan 2. This group comprising 12% of the pan's production emerged only 44.4% compared to a range of 86.6 to 94.2% for the other groups.

To evaluate performance of males mating tests were set up with individual cages containing 1 male and 15 females. Thirty-five males each from late pupation groups from pans 1, 3, and 5 were used. Females for all groups received a nonlimited amount of medium and were of average size. Females were egged and the test terminated at 8 days.

Both survival and mating records indicated a direct relationship between quantity of food provided and quality of the insects. Since mortality rates of males, obtained with limited feeding of the larvae increased more rapidly with age than the controls, it is expected that a greater spread in theoretical matings in favor of the control would have been obtained in a longer test period of 12 to 14 days involving 25 females per cage. This test does not indicate the feasibility of limiting larval consumption to produce small flies. However, by careful control of the rearing process to prevent discarding unused medium and limiting exposure of medium to incubation before consumption 1 pound of meat should produce a minimum of 1,000 healthy, vigorous flies or a ratio of 1 pound of larvae for 6 pounds meat consumed.

d. Preliminary test with polyethylene sheets in rearing
procedure (Graham, Dudley)

In an effort to reduce the amount of labor required in cleaning the rearing vats, sheets of polyethylene were placed over the bottom of the rearing vats before they were filled with medium. The polyethylene used was of 3 mil.

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In an effort to reduce the amount of labor required in cleaning the rearing vats, sheets of polyethylene were placed over the bottom of the rearing vats before they were filled with medium. The polyethylene used was of 3 mil.

thickness and clear. Though not 100 percent in effectiveness the sheets greatly reduced the amount of cleaning required. The sheets used cost about 35 cents each. In larger quantities this cost would be greatly reduced.

B. Influence of egging method on larval-rearing efficiency
(Baumhover, Graham, New)

During routine rearing for colony it was noted that when flies were egged with attractants as developed by Mr. Graham, not only were larger larvae produced in some instances, but greater recovery realized than in the average rearing procedure. Consequently pans in duplicate (10,000 eggs each), were reared through using larvae from eggs obtained with (1) incubated heart juice and (2) larval-medium, compared to the standard vial method. Results of this test are tabulated below:

Pan No.	Material	: Liters : : medium : : used :	: Pounds : : meat : : used :	: cc's. : : pupae : : produced :	: No. of : : pupae : : per cc. :	: Total : : No. of : : pupae :	: Pounds : : meat : : per 1,000 : : larvae :	: Average : : Wt. of : : larvae : : in mg. :
1	Vial	12.9	11.4	735	7.6	5,606	2.04	77.6
2	do.	12.9	11.4	795	7.7	6,121	1.86	76.6
3	Incubated heart juice	11.4	10.0	995	7.4	7,363	1.34	86.0
4	do.	11.4	10.0	995	7.3	7,263	1.38	83.8
5	Larval-medium	11.4	10.0	1,015	7.4	7,511	1.33	80.4
6	do.	11.4	10.0	865	7.5	6,487	1.54	79.6

Further testing is required to determine if:

(1) Forcing oviposition in vials is harmful;

(2) If the attractant induces oviposition of only the more vigorous females, or,

thickness and clear. Though not 100 percent in effectiveness the sheets greatly reduced the amount of cleaning required. The sheets used cost about 35 cents

each. In larger quantities this cost would be greatly reduced.

B. Influence of egg rearing method on larval-rearing efficiency (Baumhauer, Graham, New)

During routine rearing for colony it was noted that when flies were egged with attractants as developed by Mr. Graham, not only were larger larvae produced in some instances, but greater recovery realized than in the average rearing procedure. Consequently pans in duplicate (10,000 eggs each), were reared through using larvae from eggs obtained with (1) incubated heart juice and (2) larval-medium, compared to the standard vital method. Results of this test are tabulated below:

No.	Material	used	used	per cc.	pupae	larvae	in mg.
Pan	medium	meat	pupae	meat	No. of	larvae	
Liters	Pounds	cc's.	No. of	Total	meat	Wt. of	
Average							
1	Vital	12.9	11.4	735	7.6	5,606	2.04
2	do.	12.9	11.4	795	7.7	6,121	1.86
3	Incubated heart juice	11.4	10.0	995	7.4	7,363	1.34
4	do.	11.4	10.0	995	7.3	7,263	1.38
5	Larval-medium	11.4	10.0	1,015	7.4	7,511	1.33
6	do.	11.4	10.0	865	7.5	6,487	1.54

Further testing is required to determine if:

(1) Forcing oviposition in vials is harmful;

(2) If the attractant induces oviposition of only the more

vigorous females, or,

- (3) Whether the attractant is supplying beneficial materials either directly through transfer of small quantities of the attractant with the eggs or indirectly through females feeding on the materials.

C. Test for efficiency of eggging with unused medium (Graham, Dudley)

Flies emerging from 100 cc. pupae in each of five colony cages were eggged using attractive media when 8 1/2 days old. The media was placed in pint jars and surrounded with warm water. After two hours the test was terminated. Total oviposition was 292,000 eggs or an average of 58,400 per cage. These flies were eggged in the same manner again the following day and laid an average of 2,000 eggs per cage; number of eggs calculated on weight basis of 20 eggs to each mg.

D. Preliminary test of efficiency of large cage vs. standard colony fly cage
(Graham, Dudley, Baumhover)

A cage 72" x 72" x 44" was constructed with a rearing area equal to 60 standard colony cages. The sides and top are screen wire. Wrapping paper hung from a rack near the top greatly increased the resting area.

The cage was stocked with approximately 45,000 flies and eggged at eight days of age. Eggs were obtained by placing attractive larval-rearing medium in 3 strips on tin foil fitted over a double flourescent light. Approximately 2,100,000 eggs were laid in 8 hours by this procedure. The eggging device was not large enough to accommodate all the flies at one time and the eggging medium dried out before all the flies could lay. By comparison, 60 small cages stocked with as many flies would give about 3,000,000 eggs.

(3) Whether the attractant is supplying beneficial materials either

directly through transfer of small quantities of the attractant

with the eggs or indirectly through females feeding on the

materials.

C. Test for efficiency of egg-laying with unused medium (Graham, Dudley)

Flies emerging from 100 cc. pupae in each of five colony cages were

egged using attractive media when 8 1/2 days old. The media was placed in pint

jars and surrounded with warm water. After two hours the test was terminated.

Total oviposition was 292,000 eggs or an average of 58,400 per cage. These flies

were egged in the same manner again the following day and laid an average of 2,000

eggs per cage; number of eggs calculated on weight basis of 20 eggs to each mg.

D. Preliminary test of efficiency of large cage vs. standard colony
fly cage (Graham, Dudley, Baumhover)

A cage 72" x 12" x 14" was constructed with a resting area equal to 60

standard colony cages. The sides and top are screen wire. Wrapping paper hung

from a rack near the top greatly increased the resting area.

The cage was stocked with approximately 15,000 flies and egged at eight

days of age. Eggs were obtained by placing attractive larval-rearing medium in

3 strips on tin foil fitted over a double fluorescent light. Approximately

2,100,000 eggs were laid in 8 hours by this procedure. The egg-laying device was not

large enough to accommodate all the flies at one time and the egg-laying medium dried

out before all the flies could lay. By comparison, 60 small cages stocked with

as many flies would give about 3,000,000 eggs.

E. Effect of submergence on 2-day-old screw-worm pupae held in lots of 100 in pint jars containing sand (Baumhover, New)

Reports from ranchers in Florida indicate the incidence of screw-worm infestations during 1956 and up to the present date to be as great or greater than any time since the screw-worm was introduced. Rainfall during 1956 has been subnormal, resulting in less land area being flooded than normal.

Following is a quotation from the U. S. Weather Bureau's annual Florida summary for 1956:

"This was a dry year for most of the Peninsula, and for the coastal areas of the Northwest Division. Particularly dry areas were the Lower East Coast and Keys Divisions, the Tampa vicinity and southeastward to the southern limit of the Hills section, the eastern portion of the Everglades, and the coastal portions of the North and North Central Divisions. The vicinity of Blountstown was particularly rainy, resulting in annual precipitation that significantly exceeded the long-term mean. Heavy precipitation in October brought the annual totals close to the long-term means in the eastern interior of the South Central Division and a contiguous area in the southern portion of the North Central Division although the rest of the year was rather dry."

Eighty-nine percent of the 65 weather stations for which long-term means were available reported a negative departure ranging from 0.37 to 22.08 inches for 1956.

B. Effect of emergence on 2-day-old screw-worm pupae held in lots of 100 in pint jars containing sand (Bannover, New)

Reports from ranchers in Florida indicate the incidence of screw-worm infestations during 1956 and up to the present date to be as great or greater than any time since the screw-worm was introduced. Rainfall during 1956 has been subnormal, resulting in less land area being flooded than normal.

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Eighty-nine percent of the 65 weather stations for which long-term means were available reported a negative departure ranging from 0.37 to 22.08 inches for 1956.

An initial test was conducted in which 100 2-day-old screw-worm pupae were placed in pint jars, covered with 1 inch of sand and filled with sufficient tap water to provide a 1/4-inch layer of water above the sand. Lots of 3 samples each were exposed from 1 to 5 days. At the end of the exposure the pupae were removed from the sand, air dried, and placed in screen cylinders over water. All exposures showed a reduction in percent emergence. Emergence ranged from an average of 74% for the 1-day exposure to 0.3% for the 5-day exposure compared to 84% for the controls held in damp sand (40 cc. water added to 120 cc. air-dried sand) and 80% for the controls held in dry sand. Delay in emergence was approximately equal to the time submerged. Data are tabulated below:

Effect of submergence on 1 1/2-day-old (36 + 7 hours) screw-worm pupae, held in lots of 100 in pint jars containing sand at 80 + 2°F.

Group	<u>Days submerged</u>	<u>Percent emergence</u>	<u>Days emergence delayed</u>
1	1	72	1
2	1	77	1
3	1	74	1
4	2	53	2
5	2	55	2
6	2	50	2
7	3	37	3
8	3	45	3
9	3	45	3
10	4	14	5
11	4	4	5
12	4	8	5
13	5	1	-
14	5	0	-
15	5	0	-
16	Held in moist sand	81	0
17	do.	85	0
18	do.	87	0
19	Held in dry sand	82	0
20	do.	78	0
21	do.	80	0

An initial test was conducted in which 100 2-day-old screw-worm pupae were placed in pint jars, covered with 1 inch of sand and filled with sufficient tap water to provide a 1/4-inch layer of water above the sand. Lots of 3 samples each were exposed from 1 to 5 days. At the end of the exposure the pupae were removed from the sand, air dried, and placed in screen cylinders over water. All exposures showed a reduction in percent emergence. Emergence ranged from an average of 74% for the 1-day exposure to 0.3% for the 5-day exposure compared to 84% for the controls held in damp sand (40 cc. water added to 120 cc. air-dried sand) and 80% for the controls held in dry sand. Delay in emergence was approximately equal to the time submerged. Data are tabulated below:

Effect of submergence on 1 1/2-day-old (36 + 7 hours) screw-worm pupae, held in lots of 100 in pint jars containing sand at 80 + 2° F.

Group	Days submerged	Percent emergence	Days emergence delayed
1	1	72	1
2	1	77	1
3	1	74	1
4	2	53	2
5	2	55	2
6	2	50	2
7	3	37	3
8	3	45	3
9	3	45	3
10	4	14	4
11	4	4	4
12	4	8	4
13	5	11	-
14	5	0	-
15	5	0	-
16	Held in moist sand	81	0
17	do.	85	0
18	do.	87	0
19	Held in dry sand	82	0
20	do.	78	0
21	do.	80	0

This test was repeated using the same procedure but submerging pupae of various age groups. Data are contained in table 2.

Table 2.--Effect of submergence on screw-worm pupae of different ages held in lots of 100 in pint jars containing sand and tap water at $80 \pm 2^\circ\text{F}$.

Days submerged	: Percent emergence ^{1/}	:	Days submerged	: Percent emergence
<u>1 1/4-day-old pupae</u>			<u>4 1/4-day old pupae</u>	
1	59		1	94
2	36		2	91
3	14		3	71
4	3		4	60
5	0.3		5	17
<u>2 1/4-day-old pupae</u>			<u>5 1/4-day-old pupae</u>	
1	93		1	94
2	90		2	86
3	75		3	67
4	70		4	38
5	34		5	9
<u>3 1/4-day-old pupae</u>			(controls) moist sand	
1	93			87
2	91		dry sand	98
3	71		screen cylinder over	97
4	68		water	
5	51			

^{1/} Average of 3 lots of 100

Percent emergence of 1 1/4-day-old pupae was reduced similarly to that of 1 1/2-day-old pupae in the preceding test. However, reduction in percent emergence of pupae 2 1/4, 3 1/4, 4 1/4, and 5 1/4 days old before being submerged was not as great as for the younger pupae. In fact pupae from these age groups submerged 1 day emerged as well as the controls. Pupae 2 1/4 and 3 1/4 days old survived 5 days submergence better than either younger or older pupae.

This test was repeated using the same procedure but submerging pupae

of various age groups. Data are contained in table 2.

Table 2.--Effect of submergence on screw-worm pupae of different ages held in lots of 100 in pint jars containing sand and tap water at 80 + 2°F.

Days submerged		Percent emergence		Days submerged		Percent emergence	
1 1/4-day-old pupae		1 1/4-day-old pupae		2 1/4-day-old pupae		3 1/4-day-old pupae	
1	29	1	94	1	93	1	93
2	36	2	91	2	90	2	91
3	14	3	71	3	75	3	71
4	3	4	60	4	70	4	68
5	0.3	5	17	5	34	5	51

F. Ovipositional response of fertile-mated screw-worm flies compared to that of sterile-mated females (Baumhover, Graham, Dudley)

Egg-mass-collection data during the recent Clewiston, Fla. field test involving the release of sterile screw-worm flies showed a greater decline in number of egg masses collected than might be expected in view of the approximately 17% sterility rate. Dr. E. F. Knipling had suggested the possibility that females mating with sterile males may show less tendency to oviposit than fertile-mated females. Under his direction two tests were conducted in outdoor cages at the Bithlo laboratory.

In the first test larvae from egg masses collected in the Clewiston test area were placed on wounded rabbits to rear the test insects. Although 6 rabbits were used, the yield of larvae was disappointing. Only 3 rabbits survived up to 2 or 3 days. Upon death the infested hind quarters were severed from the animals and placed in pans over a small amount of water in the rearing cabinet. This method produced considerably less odor than when entire carcasses were incubated. Larvae produced were of good quality, averaging 95.0 mg. and emerging 94.0%.

Only 200 rabbit-reared females were obtained. This number was divided between two colony cages, one containing a like number of media-reared laboratory stock (BA F₁₁) males treated with 7,500 r and the other with a like number of the same culture males, untreated.

Because of the uncertainty of the weather and the unpredictable fate of the flies after release into the outdoor cages, the test insects were held for 6 days in colony cages under standard laboratory conditions. For release

F. Ovipositional response of fertile-mated screw-worm flies compared to that of sterile-mated females (Bannhovey, Graham, Bailey)

Egg-mass-collection data during the recent Cleveland, Fla. field test

Involving the release of sterile screw-worm flies showed a greater decline in number of egg masses collected than might be expected in view of the approximately 1% sterility rate. Dr. E. F. Knipping had suggested the possibility that females mating with sterile males may show less tendency to oviposit than fertile-mated females. Under his direction two tests were conducted in outdoor cages at the Bldg. Laboratory.

In the first test larvae from egg masses collected in the Cleveland

test area were placed on wounded rabbits to rear the test insects. Although 6 rabbits were used, the yield of larvae was disappointing. Only 3 rabbits survived up to 2 or 3 days. Upon death the infested hind quarters were severed from the animals and placed in pans over a small amount of water in the rearing cabinet. This method produced considerably less odor than when entire carcasses were incubated. Larvae produced were of good quality, averaging 25.0 mg. and emerging 94.0%.

Only 200 rabbit-reared females were obtained. This number was divided

between two colony cages, one containing a like number of media-reared laboratory stock (BA F₁₁) males treated with 7,500 r and the other with a like number of the same culture males, untreated. Because of the uncertainty of the weather and the unpredictable fate of the flies after release into the outdoor cages, the test insects were held for 6 days in colony cages under standard laboratory conditions. For release

the flies were captured with shell vials and the females were individually recounted as both sexes were released into the 10' x 10' x 10' outdoor cage. Mortality at this time had reduced the number of females available to 80 for each cage. Of this number five individuals in each group showed serious wing damage.

Two infested goats were placed in each cage for 1 hour, removed for 1 hour and placed in the opposite cage for an additional hour. Results of the test are tabulated in the accompanying table 3.

During the second test a similar procedure was followed except that 3 goats infested naturally at the Bithlo laboratory were used to obtain insects for testing. These goats produced 812 larvae without suffering serious injury. The larvae produced ranged in size from 96.8 mg. for the first collection to 107.0 mg. for the second collection and emergence was 97.5%. Larvae were collected by confining the goats in a large watering trough containing a grate placed over sand. Females emerging over a 24-hour period were used, with 300 individuals being available. At 6 days when the flies, mated as in the previous test, were released into the outdoor cage, 10% mortality had occurred, yielding 135 females for each cage. During the last 3 days prior to release these flies were held in an unlighted room to limit wing damage.

During both tests food and water provided in the holding cages were transferred to the outdoor cages. However, after release into the outdoor cages the flies did not live well. In the first test very few flies were alive on the third day following release, while in the second test most of the flies had died at 5 days.

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Table 3.--Screw-worm egg-mass collections from infested goats held in 10' x 10' x 10' outdoor screened cages containing fertile-mated and sterile-mated flies.

Date (1957)	: Tempera- : ture : in C°.	Time goats exposed		Normal-mated females		Sterile-mated females	
		In	Out	Goat	: Egg : masses	Goat	: Egg : masses

Test No. 1, 80 females in each group

1/7	27.0	12:30	1:30	II & IV	15	I & III	16
		2:30	3:30	I & III	22	II & IV	14
1/8	27.5	12:30	1:30	II & IV	1	I & III	8
		2:30	3:30	I & III	1	II & IV	4
1/9	27.0	12:30	1:30	II & IV	<u>0</u>	I & III	<u>0</u>
Total masses					39		42
Masses hatching					34		0

Test No. 2, 135 females in each group

2/11	30.0	11:10	12:10	III	3	I	4
				IV	5	II	47
	30.0	1:10	2:10	I	27	III	9
				II	0	IV	21
2/12	23.5	11:20	12:20	III	32	I	6
				IV	26	II	1
	22.0	1:20	2:20	I	9	III	0
				II	10	IV	1
2/13	22.0	11:40	12:40	I	4	II	2
				III	2	IV	19
		1:40	2:40	II	2	I	6
				IV	6	III	0
2/15	25.0	11:30	12:30	I & III	<u>0</u>	II & IV	<u>0</u>
Total masses					126		116
Masses hatching					117		2

Table 3.--Screw-worm egg-mass collections from infested goats held in 10' x 10' x 10' outdoor screened cages containing fertile-mated and sterile-mated flies.

(Date)	in C°.	In	Out	Goat	masses	Goat	masses	egg	egg
Date	time	exposed	exposed	Normal-mated females	Sterile-mated females	Normal-mated females	Sterile-mated females	egg	egg

Test No. 1, 80 females in each group

1/7	27.0	12:30	1:30	II & IV	15	I & III	14
		2:30	3:30	I & III	22	II & IV	14
1/8	27.5	12:30	1:30	II & IV	1	I & III	8
		2:30	3:30	I & III	1	II & IV	4
1/9	27.0	12:30	1:30	II & IV	0	I & III	0
					32		12
				Total masses	34		0
				Masses hatching			

Test No. 2, 135 females in each group

2/11	30.0	11:10	12:10	III	3	I	4
				IV	5	II	14
	30.0	1:10	2:10	I	27	III	9
				II	0	IV	21
2/12	23.5	11:20	12:20	III	32	I	6
				IV	26	II	1
	22.0	1:20	2:20	I	9	III	0
				II	10	IV	1
2/13	22.0	11:40	12:40	I	4	II	2
				III	2	IV	13
		1:40	2:40	II	2	I	6
				IV	6	III	0
2/15	22.0	11:30	12:30	I & III	0	II & IV	0
					126		116
				Total masses	117		2
				Masses hatching			

Results and discussion

In test No. 1 the normal-mated females laid 39 masses (determined by weight--12.5 mg. equal 1 mass or 250 eggs) compared to 42 for the sterile-mated females. In test No. 2, 126 egg masses were laid by normal-mated females compared to 116 for the sterile-mated females. Although these 2 tests indicate no significant difference in ovipositional response of the 2 groups of females held under these test conditions, the possibility remains that a difference may exist in the tendency of the 2 groups of females to travel great distances to find a host. In a field test P^{32} labeled animal-reared females might be released after being mated with normal and sterile males.

Here, again, provision for natural conditions would be thwarted to some extent by the necessity for holding the flies under cage conditions until a very high percentage of the females had been mated.

The 2 fertile egg masses recorded in test No. 2 from the sterile-mated group are interpreted as accidental inclusion of a fertile male during sexing, or that wild females in the test area may have followed the infested goats into the cage.

G. Adult screw-worm feeding tests (Baumhover, New)

Two tests were conducted to study the influence of various feeding methods on adult longevity, oviposition, and pupal production.

In the first test (table 4) duplicate colony cages were set up with 66 cc. of pupae each, yielding from 450 to 500 flies per cage. Various combinations of ground meat and honey and the substitution of both sugar cubes and black strap molasses for honey were tested. One replicate of each feeding method was egged with an attractant, incubated larval medium in pint jars at

Results and discussion

In test No. 1 the normal-mated females laid 39 masses (determined by weight--12.5 mg. equal 1 mass or 250 eggs) compared to 12 for the sterile-mated females. In test No. 2, 120 egg masses were laid by normal-mated females compared to 110 for the sterile-mated females. Although these 2 tests indicate no significant difference in ovipositional response of the 2 groups of females held under these test conditions, the possibility remains that a difference may exist in the tendency of the 2 groups of females to travel great distances to find a host. In a field test³² labeled animal-reared females might be released after being mated with normal and sterile males.

Here, again, provision for natural conditions would be thwarted to some extent by the necessity for holding the flies under cage conditions until a very high percentage of the females had been mated.

The 2 fertile egg masses recorded in test No. 2 from the sterile-mated group are interpreted as accidental inclusion of a fertile male during sexing, or that wild females in the test area may have followed the infested goats into the cage.

G. Adult screw-worm feeding tests (Barnhaver, New)

Two tests were conducted to study the influence of various feeding methods on adult longevity, oviposition, and pupal production. In the first test (table 4) duplicate colony cages were set up with 60 cc. of pupae each, yielding from 450 to 500 flies per cage. Various combinations of ground meat and honey and the substitution of both sugar cubes and black strap molasses for honey were tested. One replicate of each feeding method was egged with an attractant, incubated larval medium in pint jars at

37° to 39°C. and the other in vials held at 35° to 37°C. The vial method produced from 2 to 4 times as many eggs per cage as the attractant, however, the viability of eggs obtained with the attractant was uniformly good regardless of the feeding method, in contrast to only 15% and 30% hatch for some of the cages egged with vials.

Cages 3 and 4 represented the standard feeding method of a meat-honey mixture replaced after the 3rd day with honey alone. Retention of the former during the 8-day period showed no advantage except for the cage egged with attractant. Flies fed sugar cubes alone laid few eggs although the viability was good. Flies fed meat-honey replaced on the 3rd day with sugar cubes laid well in vials, but viability was only 15%. Flies fed black strap molasses alone or in combination with ground meat lived well but egg yield was low and except for eggs obtained with attractant, viability was low.

Several groups were selected for a comparison of pupal production from the eggs obtained. Eggs obtained with the attractant from flies fed meat-honey plus honey after the 3rd day showed a 73% pupal recovery and an average requirement of only 0.83 pound meat per 1,000 for 2 pans. Pupae reared from flies fed meat-honey plus sugar cubes after the 3rd day and egged with attractant compared favorably with the above group. However, eggs obtained from these same groups by the vial method yielded somewhat less than half as many pupae and required almost twice as much meat plus 1,000 pupae. Eggs obtained by the vial method from flies fed honey only, gave the lowest pupal recovery, 24%.

37° to 39°C. and the other in vials held at 35° to 37°C. The vial method produced from 2 to 4 times as many eggs per cage as the attractant, however, the visibility of eggs obtained with the attractant was uniformly good regardless of the feeding method, in contrast to only 15% and 30% hatch for some of the cages egged with vials.

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Table 4.--Influence of various adult screw-worm feeding methods on oviposition as induced by attractant and vial methods with a comparison of pupae reared from selected groups.

Cage:	Food supplied in addition to tap water ^{1/}	Egg production						Pupal production from 375 mg. of eggs			
		Percent mortality		mg. eggs obtained ^{2/}		Vials		:(7,500) per pan--2 pans each group			
		at 18 days		Attrac-		:Avg. per		Percent hatch		Pounds meat	
		Male	Female	tant	Total	female	:	meat	Number	per cc.	per 1,000
1	Honey	--	--	400	--	--	99				
2	do.	4.9	2.6	--	1,560	7.1	86	(a 4.32	1,461	7.9	2.96
								(b 4.32	2,171	8.1	1.99
3	Meat-honey replaced after 3rd day with honey	--	--	660	--	--	97				
4	do.	4.0	6.7	--	2,110	11.6	97	(a 4.32	3,315	8.5	1.30
								(b 4.77	3,397	8.6	1.40
5	Meat-honey entire period + honey after 3rd day	--	--	1,120	--	--	98	(a 4.55	5,738	9.3	0.79
								(b 4.55	5,248	8.2	0.87
6	do.	2.4	3.2	--	2,340	11.1	94	(a 4.32	2,485	8.2	1.74
								(b 4.32	3,157	8.2	1.37
7	Fresh meat on alternate days	--	Discontinued due to high mortality								
8	do.	--	Discontinued due to high mortality								
9	Fresh meat on alternate days + honey after 3rd day	--	--	300	--	--	99				
10	do.	12.2	9.2	--	1,470	7.5	30				
11	Sugar cubes	--	--	400	--	--	92				
12	do.	8.8	13.7	--	720	3.9	93				

Table 4 (continued)

		Egg production					Pupal production from 375 mg. of eggs					
		Percent mortality:		mg. eggs obtained ^{2/}		Vials		(7,500) per pan--2 pans each group				
Cage:	Food supplied in	at 18 days	Attrac-	Avg. per	Percent hatch	Pounds meat	Number per cc.	Pounds meat				
No.::	addition to tap	Male	Female	tant	Total	female	hatch	meat	Number	per 1,000		
	water ^{1/}											
13	Meat-honey replaced after 3rd days with sugar cubes	--	--	3/	--	--	--					
14	do	7.0	6.2	--	1,420	7.9	15					
15	Meat-honey entire period + sugar cubes after 3rd day	--	--	1,300	--	--	97	(a 4.55 (b 4.55	4,928 4,640	8.6 8.3 0.92 0.98		
16	do.	7.2	10.3	--	2,080	10.4	77	(a 4.32 (b 4.32	1,974 3,116	8.4 8.2 2.19 1.38		
17	Black strap molasses	--	--	330	--	--	94					
18	do.	20.2	12.5	--	730	3.7	30					
19	Meat-black strap molasses entire period + black strap molasses after 3rd day	--	--	80	--	--	99					
20	do.	12.6	10.3	--	220	1.4	68					

1/ Meat-honey and meat-molasses combinations in ratio of 1.5:1

2/ Average mg. eggs per female bases on number surviving at 8 days; flies exposed to attractant 1 1/2 hours

3/ Flies escaped

adjuvants

yesb brc + enqes after

after 36 hrs
black string
see entire paper +

3/ After escape

Table 5 contains data on the second adult feeding test in which various meat-honey combinations were tested along with substitution of sugar cubes for honey and bovine serum for meat. In this test duplicate cages were set up as in the first test, however, each of the duplicates were first egged with the incubated larval medium exposed for 3 1/2 hours and the following day re-egged with vials. Mortality was based on flies dead following egging with attractant which may in part account for the higher mortality compared to the first test. Average mg. of eggs per female was based on the original number of females emerged. However, observation of the cages before and after egging with attractant did not indicate an appreciable increase in mortality before the counts were made.

Egg recovery for all groups was good ranging from a low of 8.8 mg. per female for flies fed honey alone to a high of 13.4 for females feeding on meat-honey the entire period plus honey after the 3rd day. Percent hatch ranged from 94% to 100% based on eggs obtained in vials. Previous observations indicated that flies feeding on incubated larval medium derived some benefit from it, possibly accounting for egg viability being somewhat higher than for eggs normally obtained in vials containing fresh meat, particularly from flies receiving no animal protein.

Unfortunately hatching records for eggs obtained with the attractant were lost in this test due to a temperature rise to 44°C. as the attractant material dried out over the extended egging period of 3 1/2 hours.

Larvae were reared from a 375 mg. sample of eggs from each duplicate. Pupal recovery was greatest for the meat-honey plus sugar cubes the entire period (64%) and for the 3:1 bovine serum-honey mixture (63%).

Table 2 contains data on the second adult feeding test in which various meat-honey combinations were tested along with substitution of sugar cubes for honey and bovine serum for meat. In this test duplicate cages were set up as in the first test, however, each of the duplicates were first egged with the incubated larval medium exposed for 3 1/2 hours and the following day re-egged with vials. Mortality was based on flies dead following egging with attractant which may in part account for the higher mortality compared to the first test. Average mg. of eggs per female was based on the original number of females emerged. However, observation of the cages before and after egging with attractant did not indicate an appreciable increase in mortality before the counts were made. Egg recovery for all groups was good ranging from a low of 8.8 mg. per female for flies fed honey alone to a high of 13.4 for females feeding on meat-honey the entire period plus honey after the 3rd day. Percent hatch ranged from 94% to 100% based on eggs obtained in vials. Previous observations indicated that flies feeding on incubated larval medium derived some benefit from it, possibly accounting for egg viability being somewhat higher than for eggs normally obtained in vials containing fresh meat, particularly from flies receiving no animal protein. Unfortunately hatching records for eggs obtained with the attractant were lost in this test due to a temperature rise to 44°C. as the attractant material dried out over the extended egging period of 3 1/2 hours. Larvae were reared from a 375 mg. sample of eggs from each duplicate. Pupae recovery was greatest for the meat-honey plus sugar cubes the entire period (63%) and for the 3:1 bovine serum-honey mixture (63%).

Further testing is recommended comparing these feeding methods with the standard for several successive generations to obtain more conclusive data.

Although the honey-meat mixture plus honey alone the entire period did not show an advantage in eggs produced or pupal recovery over the standard in which the meat-honey was replaced after the 3rd day with honey alone, the former method has been temporarily adopted to simplify care of the adults. With this method the cage of flies requires no attention from the time it is set up until used. Previously the meat-honey mixture was removed on the 4th day to prevent oviposition on this material. However, in no instance have eggs been observed on this material when honey alone is also present.

Further testing is recommended comparing these feeding methods with the standard for several successive generations to obtain more conclusive data. Although the honey-meat mixture plus honey alone the entire period did not show an advantage in eggs produced or pupal recovery over the standard in which the meat-honey was replaced after the 3rd day with honey alone, the former method has been temporarily adopted to simplify care of the adults. With this method the cage of flies requires no attention from the time it is set up until used. Previously the meat-honey mixture was removed on the 15th day to prevent oviposition on this material. However, in no instance have eggs been observed on this material when honey alone is also present.

Tab 5.--Influence of various adult screw-worm feeding methods with a comparison of pupae reared from each group.

		Egg Production						Pupal production from 375 mg. eggs			
		Percent		mortality		mg. eggs obtained ^{2/}		(7,500) obtained with vials--		1 pan each group	
Cage No.:	Food supplied in addition to tap water ^{1/}	at 9 days	Attrac-	Avg. per	Percent	Number	Pounds meat	Percent	emergence		
		Male	Female	tant	Vials	female	hatch ^{3/}	Number	per cc.	per 1,000 ^{4/}	
1	Honey	14	28	1,220	390	8.8	100	2,730	8.2	2.2	93.1
2	do.	21	16	1,112	620	9.8	100	3,360	8.4	1.8	93.5
3	Meat-honey replaced after 3rd day with honey	22	28	1,380	430	12.7	97	3,782	8.5	1.6	93.7
4	do.	22	14	1,230	680	11.4	100	4,132	8.7	1.5	93.5
5	Meat-honey entire period + honey after 3rd day	13	24	2,150	400	13.4	100	3,958	8.7	1.5	97.5
6	do.	43	31	1,770	370	11.6	97	4,191	8.3	1.5	94.8
7	Meat-honey + honey entire period	21	14	1,960	670	12.8	100	3,760	8.0	1.6	95.8
8	do.	41	49	1,340	280	9.9	100	3,483	8.1	1.8	98.6
9	Meat-honey + sugar cubes after 3rd day	21	21	1,700	250	11.1	97	3,440	8.0	1.8	92.9
10	do.	16	22	1,160	450	10.9	94	4,578	8.4	1.3	97.9
11	Meat-honey + sugar cubes entire period	13	14	1,570	610	11.1	100	4,887	8.5	1.2	96.7
12	do.	11	11	1,370	840	11.1	100	4,704	8.4	1.3	96.8
13	Bovine serum-honey 1:1 entire period	21	25	1,110	540	9.1	97	3,157	8.2	1.9	96.2
14	do.	24	14	1,120	1,090	11.6	100	3,654	8.4	1.7	95.5
15	Bovine serum-honey 3:1 entire period	12	16	1,120	930	11.0	97	4,551	8.2	1.3	96.1
16	do.	20	25	1,000	440	10.4	100	4,914	8.4	1.2	92.8

1/ Meat-honey combinations in ratio of 1.5:1 by volume.

2/ Flies were exposed to attractant 1.5:30 to 19:00 on the 8th day and then egged in vials the following day; mg. eggs per female based on original number in cage.

3/ Based on eggs obtained in vials

4/ Pounds meat 6.1 for all pans.

H. Sterility of irradiated (7,500 r) female screw-worm flies in relation to age after treatment with notes on normal female longevity (Baumhover, New)

Six colony cages of 300 females each, treated with 7,500 r on the 5th day of pupal development were mated with normal males in the ratio of 1 male to 5 females. At 8 and 20 days of adult life the surviving females were egged.

On the 8th day 11 abnormal eggs were obtained with none being oviposited on the 20th day. Although 163 females and 13 males survived 20 days none were alive on the 28th day and the test was terminated. The ratio of 1 male to 5 females was used to increase the life expectancy of the females. In a previous mating test not reported due to high female mortality in some of the cages, it was observed that when equal numbers of males and females were caged together female mortality was much higher than when females of the same culture were caged alone. At 12 days 8 cages of 100 females each caged with a like number of males averaged 89% mortality compared to 25% mortality for 4 cages of 100 females each caged alone. These females were first generation flies reared from egg mass collections made in the Clewiston, Fla. test area. The males were from the Bithlo "A" strain. However, 2 cages of the Bithlo "A" strain females, although caged with equal numbers of males averaged only 20% mortality at 12 days, indicating a more vigorous laboratory strain and/or a difference in adaptation to laboratory conditions.

I. Fly release containers (Baumhover, New)

1. Cardboard carton

Recently Dr. Lindquist discussed the desirability of increasing the number of flies per release carton from 100 to 800 with a minimum increase in size of the box. The Clewiston field test was conducted, placing 100 flies

H. Sterility of irradiated (7,500 r) female screw-worm flies in relation to age after treatment with notes on normal female longevity (Bamhovey, New)

Six colony cages of 300 females each, treated with 7,500 r on the 5th day of pupal development were mated with normal males in the ratio of 1 male to 5 females. At 8 and 20 days of adult life the surviving females were aged. On the 8th day 11 abnormal eggs were obtained with none being oviposited on the 20th day. Although 153 females and 13 males survived 20 days none were alive on the 28th day and the test was terminated. The ratio of 1 male to 5 females was used to increase the life expectancy of the females. In a previous mating test not reported due to high female mortality in some of the cages, it was observed that when equal numbers of males and females were caged together female mortality was much higher than when females of the same culture were caged alone. At 12 days 8 cages of 100 females each caged with a like number of males averaged 89% mortality compared to 25% mortality for 4 cages of 100 females each caged alone. These females were first generation flies reared from egg mass collections made in the Glaston, Fla. test area. The males were from the Bitho "A" strain. However, 2 cages of the Bitho "A" strain females, although caged with equal numbers of males averaged only 20% mortality at 12 days, indicating a more vigorous laboratory strain and/or a difference in adaptation to laboratory conditions.

(Bamhovey, New)

I. Fly release containers

1. Cardboard carton

Recently Dr. Lindquist discussed the desirability of increasing the number of flies per release carton from 100 to 800 with a minimum increase in size of the box. The Glaston field test was conducted, placing 100 flies

as pupae in 4" x 5" x 1 1/2" waxed Klik-lok boxes as used in the frozen food industry. In a single test 829 pupae were placed in a cardboard box 4 1/2" x 5 1/2" x 2 1/2" three days before emergence. A separator was made of cardboard, similar to the folding separators used in egg crates, to provide 20 compartments. A total of 202 sq. in. of resting space was available for the flies. Notches were made in the compartments to allow free movement of the flies throughout the box. Twenty-four hours after emergence had begun (or 12 hours after completion) the flies were released by cutting a single hole in one edge of the box. General appearance of the flies was good with only 7 dead, 10 weak, and 10 deformed. Emergence was 95.1% yielding 788 individuals.

The local representative of the Container Corporation of America was contacted to obtain samples of similar containers and cost figures on lease or sale of automatic loading and folding machines. A small unit recommended by a representative of the firm would cost \$750.00 for the standard 2-year lease. This machine would automatically set up the cartons but closing would be done manually.

Six experimental cartons (No. 20057) were received for testing. Three of these were perforated at 1/4" intervals with a 1/16" drill to obtain additional aeration. Each of the cartons was loaded with sufficient pupae to produce approximately 800 flies. They were then placed in colony cages to capture any insects that might escape. After emergence was 95% complete the flies were released from the cartons. In all cartons all but 1% to 2% of the flies appeared to be vigorous flyers and readily flew from the carton. Only 2 flies out of the entire 6 containers had managed to escape from the cartons and the carton construction prevented wedging of the flies. Emergence in both

as pupae in 1" x 5" x 1 1/2" waxed Klik-Lok boxes as used in the frozen food industry. In a single test 829 pupae were placed in a cardboard box 1 1/2" x 5" x 1 1/2" three days before emergence. A separator was made of cardboard, similar to the folding separators used in egg crates, to provide 20 compartments. A total of 202 sq. in. of resting space was available for the flies. Notches were made in the compartments to allow free movement of the flies throughout the box. Twenty-four hours after emergence had begun (or 12 hours after completion) the flies were released by cutting a single hole in one edge of the box. General appearance of the flies was good with only 7 dead, 10 weak, and 10 deformed. Emergence was 95.1% yielding 788 individuals.

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types of cartons was 90%. This test indicated this type of carton to be satisfactory without additional aeration. Cost of this type of carton including the separators would be approximately \$10.00 per thousand in large quantities.

2. Aluminum unit (Methods Improvement)

An aluminum dispersal unit containing 3 compartments 4" x 4" x 1 1/2" was received from Methods Improvement for testing. Instructions were to place 800 pupae in each of the 3 compartments which varied in construction as follows:

- A. Screen walls and 2 screen baffles
- B. Smooth walls and 2 screen baffles
- C. Smooth walls and 2 smooth baffles

Pupae forming over a 6-hour period were placed in the compartments 2 days before emergence and held at 80°F. As received, the unit had two open sides. For the test one side was closed with a sheet of glass and the other with fly screen. Twenty-four hours after emergence was complete it was obvious that excessive overcrowding had occurred. Less than 4 percent of the insects were capable of flying when released and mortality ranged as high as 61 percent. Data from this test are tabulated below:

<u>Section</u>	<u>No. of flyers</u>	<u>No. dead.</u>	<u>No. emerged</u>	<u>No. not emerged</u>
A.	31	396	800	97
B.	26	422	785	97
C.	19	462	757	87

In the following test the number of pupae per compartment was reduced approximately 50 percent with the following results:

types of cartons was 90%. This test indicated this type of carton to be

satisfactory without additional aeration. Cost of this type of carton included

the separators would be approximately \$10.00 per thousand in large quantities.

2. Aluminum unit (Method Improvement)

An aluminum dispersal unit containing 3 compartments 14" x 14" x 1 1/2"

was received from Method Improvement for testing. Instructions were to place

800 pupae in each of the 3 compartments which varied in construction as follows:

A. Screen walls and 2 screen baffles

B. Smooth walls and 2 screen baffles

C. Smooth walls and 2 smooth baffles

Pupae forming over a 6-hour period were placed in the compartments

2 days before emergence and held at 80°F. As received, the unit had two open

sides. For the test one side was closed with a sheet of glass and the other

with fly screen. Twenty-four hours after emergence was complete it was obvious

that excessive overcrowding had occurred. Less than 1 percent of the insects

were capable of flying when released and mortality ranged as high as 61 percent.

Data from this test are tabulated below:

Section	No. of flyers	No. dead.	No. emerged	No. not emerged
A.	31	396	800	97
B.	26	422	782	97
C.	19	462	757	87

In the following test the number of pupae per compartment was reduced

approximately 50 percent with the following results:

<u>Compartment</u>	<u>No of flyers</u>	<u>No. dead</u>	<u>No. emerged</u>	<u>No. not emerged</u>
A	288	16	324	25
B	305	6	323	39
C	272	37	343	32

In tests conducted by Drs. R. C. Bushland and D. W. Jenkins air movement in excess of 60 m.p.h. was required to dislodge screw-worm adults from the wire screen. Consequently this material was eliminated from units further tested.

To increase area available for the flies instructions were received to hold newly emerged but hardened flies in the container for 24 hours. When 800 pupae were placed in the 4" x 4" x 1 1/2" units approximately 25 percent of the volume was required for the pupae. However, this procedure did not eliminate excessive crowding:

<u>No. flies</u>	<u>No. dead plus nonflyers</u>
800	518
600	296
400	62

Further tests involved a comparison of 4" x 4" x 1 1/2" compartment (2 baffles) with 6" x 6" x 2" compartments (3 baffles) held under several lighting and temperature conditions. Hardened flies were held 24 hours in these containers before observations were made:

Compartment	No of flies	No. dead	No. emerged	No. not emerged
A	288	16	324	22
B	302	6	323	32
C	272	27	313	32

In tests conducted by Drs. R. C. Bushland and D. W. Jenkins air move-

ment in excess of 60 m.p.h. was required to dislodge screw-worm adults from the wire screen. Consequently this material was eliminated from units further tested.

To increase area available for the flies instructions were received to hold newly emerged but hardened flies in the container for 24 hours. When 800 pupae were placed in the $1\frac{1}{2}$ " x $1\frac{1}{2}$ " x $1\frac{1}{2}$ " units approximately 25 percent of the volume was required for the pupae. However, this procedure did not eliminate excessive crowding:

No. flies	No. dead plus nonflyers
800	218
600	226
400	62

Further tests involved a comparison of $1\frac{1}{2}$ " x $1\frac{1}{2}$ " x $1\frac{1}{2}$ " compartment

(2 baffles) with $6\frac{1}{2}$ " x $6\frac{1}{2}$ " x 2" compartments (3 baffles) held under several lighting and temperature conditions. Hardened flies were held 24 hours in these containers before observations were made:

<u>Holding conditions</u>	<u>No. flies</u>	<u>No. dead plus nonflyers</u>	
		<u>4" x 4" x 1 1/2"</u>	<u>6" x 6" x 2"</u>
80°F.--Daylight	800	242	69
	600	169	--- 1/
	400	90	---
80°F.--Dark (in closed cardboard cartons)	800	140	---
	600	109	---
	400	20	---
60°F.--Dark (in refrigeration unit)	800	119	10
	600	38	12
	400	20	20

1/ Insufficient flies available

In the 4" x 4" x 1 1/2" compartments both darkness and reduction of the holding temperature to 60°F. were somewhat beneficial in reducing the number of dead or damaged flies. Unfortunately insufficient flies were available to make a more complete comparison between the 4" and 6" units, however, the 6" unit held 800 flies very well at 60°F. but was somewhat less than satisfactory at 80°F. in daylight.

Since the cardboard units with cardboard compartments worked so well, cardboard separators were substituted for the metal baffles in the 4" and 6" metal units with the following results:

<u>No. flies</u>	<u>No. dead plus nonflyers</u>
<u>4" x 4" x 1 1/2"</u>	
499	206
388	128
290	37

<u>No. dead plus nonflyers</u>	<u>No. flies</u>	<u>Holding conditions</u>
20	800	80°F.--Daylight
12	600	
10	400	
10	800	80°F.--Dark (in closed cardboard cartons)
10	600	
20	400	
10	800	60°F.--Dark (in refrigerator unit)
12	600	
20	400	

1/ Insufficient flies available

In the 1/2" x 1/2" x 1/2" compartments both darkness and reduction of the holding temperature to 60°F. were somewhat beneficial in reducing the number of dead or damaged flies. Unfortunately insufficient flies were available to make a more complete comparison between the 1/2" and 6" units, however, the 6" unit held 800 flies very well at 60°F. but was somewhat less than satisfactory at 80°F. in daylight.

Since the cardboard units with cardboard compartments worked so well, cardboard separators were substituted for the metal baffles in the 1/2" and 6" metal units with the following results:

No. dead plus nonflyers

1/2" x 1/2" x 1/2"

206	129
128	388
37	290

<u>6" x 6" x 2"</u>	
611	15
478	20
309	15
<u>Cardboard container</u>	
645	15

Although sufficient pupae were placed in the compartments to yield approximately 800, 600, and 400 in one compartment each of the 2 metal units and 800 in the cardboard unit poor emergence of this culture (73.5% for the control in screen cylinder) resulted in a reduced number of flies. In this test the 6" x 6" x 2" compartment with cardboard separators performed as well as the cardboard container, however, the cardboard separators did not improve the holding conditions in the 4" x 4" x 1 1/2" compartments.

In the event that hardened adults would be transferred to release units (to save space occupied by empty pupal cases) an emergence chamber would be required. Instructions were received to place 6,400 pupae in a 12" triangular device 12" long containing paper toweling for additional resting area. In the initial test this unit supported the number of flies with low mortality or damage.

However, in a release unit sufficiently large (6" x 6" x 2") to support 800 flies the 100 cc. of pupae required would occupy only 8.5% of the space. Since it is doubtful that hardened flies would require much less space than newly emerged flies it would not seem likely that the extra equipment and manipulation required to transfer hardened flies to release units would be justified.

Justified.

manipulation required to transfer hardened flies to release units would be newly emerged flies it would not seem likely that the extra equipment and Since it is doubtful that hardened flies would require much less space than 800 flies the 100 cc. of pupae required would occupy only 8.5% of the space. However, in a release unit sufficiently large (6" x 6" x 2") to support damage.

initial test this unit supported the number of flies with low mortality or device 12" long containing paper toweling for additional resting area. In the required. Instructions were received to place 6,400 pupae in a 12" triangular (to save space occupied by empty pupal cases) an emergence chamber would be In the event that hardened adults would be transferred to release units the holding conditions in the 14" x 14" x 1 1/2" compartments.

as the cardboard container, however, the cardboard separators did not improve test the 6" x 6" x 2" compartment with cardboard separators performed as well control in screen cylinder) resulted in a reduced number of flies. In this and 800 in the cardboard unit poor emergence of this culture (73.5% for the approximately 800, 600, and 400 in one compartment each of the 2 metal units Although sufficient pupae were placed in the compartments to yield

Cardboard container	6" x 6" x 2"
615	15
308	15
478	20
611	15

J. Ecological studies (Skipper, New, Dudley)

1. Collections in North Florida

The operation of eight status fly traps continued throughout the period. Based upon the number of adult screw-worms taken in these traps and on reports from cattlemen, screw-worm activity continued during the winter months in all areas in Florida where they had reached during the summer migration. Reports also indicate screw-worm activity in southern Georgia as late as February 1957. A tabulation of the adult screw-worms taken in status fly traps in North Florida follows:

Trap location	Number <i>C. hominivorax</i> taken per trapping period									
	:9/11-:10/2-	:10/23-:11/15-	:12/5-:12/27-	:1/8-:2/7-	:2/27-:3/20					
	:10/2	:10/23:11/15	:12/5	:12/27:1/8	:2/7	:2/27	:3/20	:4/9		
Brooksville	6	16	14	8	1	3	0	4	3	8
Ocala National Forest	2	3	14	Gate locked	5	1	67	19	Trap down	0
Trenton	33	9	Trap down	21	6	0	18	65	do.	21
Perry	73	89	11	15	39	17	25	26	2	4
Lake City	18	49	32	12	0	0	3	5	16	1
Macclenny	21	19	1	5	1	1	3	0	3	7
Yulee	3	7	1	6	0	0	1	0	0	0
Christmas	49	59	35	61	40	Trap down	21	35	Trap down	4

2. Collections in Clewiston, Fla. test area

Following the field test near Clewiston, Fla., September to December 1956, the decision was made to continue fly-trapping studies in this area in anticipation of returning for further tests the following year. Although current plans do not provide for use of this area the fly-trapping data are presented to supplement data obtained from traps in the northern half of the State. The clewiston, Fla. data are contained in table 6.

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traps in North Florida follows:

Trap location		10/2:10/23:11/15:12/5:12/27:1/8:2/7:2/27:3/20:4/9		9/11:10/2:10/23:11/15:12/5:12/27:1/8:2/7:2/27:3/20		Number C. hominivorax taken per trapping period	
Christmas	49	52	35	61	40	Trap down	4
Yulee	3	7	1	6	0	0	0
Macclenny	21	19	1	5	1	3	7
Lake City	18	49	32	12	0	3	1
Perry	73	89	11	15	39	17	4
Trenton	33	9	Trap down	21	6	0	21
Forest							
Ocala National	2	3	14	Gate	5	1	0
Brooksville	6	16	14	8	1	3	8

2. Collections in Clewiston, Fla. test area

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1956, the decision was made to continue fly-trapping studies in this area in

anticipation of returning for further tests the following year. Although current

plans do not provide for use of this area the fly-trapping data are presented

to supplement data obtained from traps in the northern half of the State. The

Clewiston, Fla. data are contained in table 6.

Table 6.--Screw-worm flies caught in liver-baited fly traps in Clewiston, Fla., test area (following termination of test) beginning December 17, 1956 and ending April 1, 1957

Trap No.:	1	2	3	4	5	W 6	E 7	E 8	K 9	10	11	12	13	14	15	Total
1	4	26	29	21	15	13	8	15	4	4	1	0	3	9	7	159
2	3	12	5	14	9	15	4	2	5	2	12	0	18	20	19	140
3	6	2	18	5	4	55	9	14	20	<u>1</u> / 33	3	1	10	27		207
4	18	12	7	2	11	14	3	16	30	26	20	2	28	46	52	287
5	116	176	105	191	131	406	188	245	78	90	53	14	46	10	14	1,863
6	5	11	2	0	<u>2</u> / ---	1	1	4	5	3	3	5	13	3	2	58
Total	152	239	166	233	170	504	213	296	142	125	122	24	109	97	121	2,714

1/ Hole in trap

2/ Trap overturned

Of particular interest are the collections made from trap No. 5 which totaled 1,863 screw-worms for the 15-week study or an average of 124 per week. With few exceptions the trapping data indicated screw-worms to be very active in this area during the winter of 1956-7.

Research Line Project ENT h2-16 Development of attractants for estimating and controlling natural screw-worm populations

A. Preliminary tests (Graham, Dudley)

Several materials other than the larval-rearing medium have been found to attract and to stimulate oviposition of gravid screw-worm flies. These are listed in order of importance:

1. Juices from thawed ground heart meat incubated at 97°F., for 36-48 hours
2. Fifteen grams of dried blood albumin and 5 grams of animal fat in 300 cc. distilled water incubated for 7 days at 97°F.
3. Fifteen grams of dried blood albumin in 300 cc. distilled water and incubated at 97°F. for 7 days
4. Four grams powdered milk in 400 cc. of hemolized blood incubated for 20 hours at 97°F.

The incubated heart juices are the most consistent in the degree of attractiveness. Large quantities of this material can be recovered when the meat thaws in preparation of the larval medium. Seventy pounds of completely thawed heart will yield a gallon of the fluid.

Preliminary tests indicate that the digestion of animal fat with dried blood albumin was more attractive than the incubated albumin alone. Various concentrations of albumin with fat were incubated in pint jars up to 10 days. These jars, with a screen on top, were placed in a cage of colony flies and observations made as to preference. All concentrations, with and without fat, showed some attractiveness. One of the fat-albumin combinations

Research Lane Project ENT 22-10 Development of attractants for estimating and controlling natural screw-worm populations

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Several materials other than the larval-rearing medium have been found to attract and to stimulate oviposition of gravid screw-worm flies. These are listed in order of importance:

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was more attractive than the others and about the same degree of attractiveness as the heart juices. Although flies did readily oviposit on the screen cover above this material they did not feed on it when the screen was removed.

The hemolized blood and powdered milk mentioned resulted in some oviposition on the screen above the jar, but was not nearly as attractive as the heart juices.

Five laboratory tests were conducted in the hexagonal cage. In 3 experiments colony flies were used and flies from natural infestations were used in the other two. Two attractants were used, and the meat portion of the attractive larval medium and the incubated heart juices.

In all experiments the control was a guinea pig with an active wound. Air temperature in the testing room was kept at 83-85°F. and the experiments were terminated after 1 1/2 hours.

Results of five experiments are tabulated as follows:

Experiment No. I with 50 colony flies 8 days old

<u>Material</u>	<u>Mode of application on/over</u>	<u>Females ovipositing</u>
Fresh rearing medium	guinea pig	2
Unused rearing medium + heart juice	do	8
Unused rearing medium	light bulb	4
Active wound (2 day)	guinea pig	8

Experiment No. II, with 100 colony flies 9 days old

<u>Material</u>	<u>Mode of application on/over</u>	<u>Females ovipositing</u>
2 parts juice - 1 part blood in cotton	guinea pig ^{1/}	1
Unused rearing medium + heart juice	guinea pig	22
Active wound (3 day)	do.	14

^{1/} Came off at beginning of experiment

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Unused rearing medium	Light bulb	4
Active wound (2 day)	Guinea pig	8

Experiment No. II, with 100 colony flies 9 days old

<u>Material</u>	<u>Mode of application on/over</u>	<u>Females ovipositing</u>
2 parts juice - 1 part blood in cotton	Guinea pig	1
Unused rearing medium + heart juice	Guinea pig	22
Active wound (3 day)	do.	14

I \ Came off at beginning of experiment

Experiment No. III, with 50 colony flies 6 1/2 days old

<u>Material</u>	<u>Mode of applica- tion on/over</u>	<u>Females ovipositing</u>
2 parts heart juice - 1 part plasma	cowhide (heated)	40
2 parts heart juice - 1 part plasma	paper (heated)	3
Active wound (4 day)	guinea pig	1

Experiment No. IV, with 33 8-day-old flies from natural infestation

<u>Material</u>	<u>Mode of applica- tion on/over</u>	<u>Females ovipositing</u>
2 parts heart juice - 1 part plasma on cotton	cowhide (heated)	0
2 parts heart juice - 1 part plasma on cotton ^{1/}	cowhide (heated)	14
2 parts heart juice - 1 part plasma on cotton	rabbit	2
Active wound (2 day)	guinea pig	9

^{1/} Around edge of hole cut through hide

Experiment No. V, with 50 8-day-old flies from natural infestation

<u>Material</u>	<u>Mode of applica- tion on/over</u>	<u>Females ovipositing</u>
2 parts heart juice - 1 part plasma on cotton	cowhide (heated)	0
2 parts heart juice - 1 part plasma on cotton with attractive medium on outside	cowhide (heated)	8
2 parts heart juice - 1 part plasma on cotton	rabbit	1
Active wound (3 day)	guinea pig	9

These tests and observations showed that the materials were more attractive on live animals or on animal skins. When these juices were put on the skin of a live animal flies came to the animal in equal or greater numbers than to an active wound.

Experiment No. III, with 50 colony flies 6 1/2 days old

<u>Material</u>	<u>Mode of applica- tion on/over</u>	<u>Females ovipositing</u>
2 parts heart juice - 1 part plasma	cowhide (heated)	40
2 parts heart juice - 1 part plasma	paper (heated)	3
Active wound (4 day)	guinea pig	1

Experiment No. IV, with 33 8-day-old flies from natural infestation

<u>Material</u>	<u>Mode of applica- tion on/over</u>	<u>Females ovipositing</u>
2 parts heart juice - 1 part plasma on cotton	cowhide (heated)	0
2 parts heart juice - 1 part plasma on cotton	cowhide (heated)	14
2 parts heart juice - 1 part plasma on cotton	rabbit	2
Active wound (2 day)	guinea pig	2

1/ Around edge of hole cut through hide

Experiment No. V, with 50 8-day-old flies from natural infestation

<u>Material</u>	<u>Mode of applica- tion on/over</u>	<u>Females ovipositing</u>
2 parts heart juice - 1 part plasma on cotton	cowhide (heated)	0
2 parts heart juice - 1 part plasma on cotton with attractive medium on outside	cowhide (heated)	8
2 parts heart juice - 1 part plasma on cotton	rabbit	1
Active wound (3 day)	guinea pig	2

These tests and observations showed that the materials were more

attractive on live animals or on animal skins. When these juices were put on the skin of a live animal flies came to the animal in equal or greater numbers than to an active wound.

Some bovine plasma was added to the attractant to make the material more desirable to feed on. Flies visiting a wound usually feed before ovipositing.

Apparatus is being developed for testing liquids in the field rather than meat. The juices seem to be as attractive as the meat and can be continuously applied with a drip system. Also, the liquid may be less attractive to secondary blowflies than the meat.

B. Cage tests (Baumhover, Bowman, Graham, Dudley)

Further attractant testing was done in cooperation with personnel of the Pesticide Chemicals Research Section. Since incubated heart juice had performed uniformly well in previous testing it was selected for more intensive study. Beginning with the source of the fresh juice, frozen ground heart allowed to thaw at air temperatures, 4 components were arbitrarily chosen:

1. Juices draining from thawing heart
2. Meat remaining from 1
3. Juices draining from thawing heart plus juices squeezed from sample
4. Meat remaining from 3

In the initial test these materials were compared, incubated for 36 hours and unincubated. Duplicate colony cages (25 females each) comparing 2 samples each were set up with the following results:

Some bovine plasma was added to the attractant to make the material

more desirable to feed on. Flies visiting a wound usually feed before

ovipositing.

Apparatus is being developed for testing lipids in the field rather

than meat. The juices seem to be as attractive as the meat and can be con-

tinuously applied with a drip system. Also, the lipid may be less attractive

to secondary blowflies than the meat.

B. Cage tests (Barnhaver, Bowman, Graham, Dudley)

Further attractant testing was done in cooperation with personnel of

the Pesticide Chemicals Research Section. Since incubated heart juice had per-

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and unincubated. Duplicate colony cages (25 females each) comparing 2 samples

each were set up with the following results:

<u>Cage</u>	<u>Material</u>	<u>Egg masses</u>
1	1a ^{1/} 2a	0 0
2	1a 2a	0 0
3	1a 3a	0 0
4	1a 3a	0 1
5	1a 4a	3 0
6	1a 4a	7 1
7	2a 3a	1 2
8	2a 3a	0 5
9	2a 4a	0 0
10	2a 4a	4 0
11	3a 4a	5 0
12	3a 4a	11 0
13	3 4	3 5
14	3 4	2 4

^{1/} "a" indicated material incubated for 36 hours at 97°F. .

<u>Cage</u>	<u>Material</u>	<u>Per masses</u>
1	1a 2a	0 0
2	1a 2a	0 0
3	1a 2a	0 0
4	1a 2a	0 1
5	1a 2a	3 0
6	1a 2a	7 1
7	2a 3a	1 2
8	2a 3a	0 2
9	2a 3a	0 0
10	2a 3a	1 0
11	2a 3a	2 0
12	2a 3a	11 0
13	3 4	3 2
14	3 4	2 1

1/2 "a" indicated material incubated for 30 hours at 20°F.

Sample 3a, incubated juice obtained by draining plus squeezing, appeared the best. However, the data were too meager to be conclusive. Consequently, the next test involved some of the many factors thought to affect ovipositional response:

<u>Cage</u>	<u>Container for sample</u>	<u>Number of flies</u>	<u>Material</u>	<u>mg. of eggs</u>
<u>Fly concentration</u>				
1	Pint jar	25	3a	195
	do.		IRM ^{1/}	0
2	do.	25	3a	155
	do.		IRM	130
3	do.	50	3a	410
	do.		IRM	110
4	do.	50	3a	190
	do.		IRM	290
5	do.	100	3a	320
	do.		IRM	860
6	do.	100	3a	610
	do.		IRM	470
<u>Shallow (cup) vs. deep (pint jar) container</u>				
7	Pint jar	25	3a	35
	Cup		3a	70
8	Pint jar	25	3a	100
	Cup		3a	0
9	Pint jar	25	IRM inc. ^{2/}	190
	Cup		do.	0
10	Pint jar	25	do.	230
	Cup		do.	0

ovipositional response:
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 Sample 3a, incubated juice obtained by draining plus squeezing.

Cage	Container for sample	Number of flies	Material	mg. of eggs
<u>Fly concentration</u>				
1	Pint jar do.	25	3a IRM	195 0
2	do. do.	25	3a IRM	155 130
3	do. do.	50	3a IRM	110 110
4	do. do.	50	3a IRM	190 290
5	do. do.	100	3a IRM	320 860
6	do. do.	100	3a IRM	610 1170
<u>Shallow (cup) vs. deep (pint jar) container</u>				
7	Pint jar Cup	25	3a 3a	35 70
8	Pint jar Cup	25	3a 3a	100 0
9	Pint jar Cup	25	IRM inc. 3a do.	190 0
10	Pint jar Cup	25	do. do.	230 0

<u>Cage</u>	<u>Container for sample</u>	<u>Number of flies</u>	<u>Material</u>	<u>mg. of eggs</u>
		<u>CO₂ anesthesia vs. no CO₂^{3/}</u>		
11	Pint jar	25 CO ₂	3a	130
	do.		LRM	130
12	do.	do.	3a	120
			LRM	140
13	do.	25 no CO ₂	3a	80
			LRM	200
14	do.	25 no CO ₂	3a	220
			LRM	30

1/ Fresh larval medium without larvae or formalin.

2/ Fresh larval medium without larvae but containing 0.1% formalin and incubated 48 hours at 37°C.

3/ Flies in cages 1 through 12 were anesthetized 2 hours before test was started flies for cages 13 and 14 were captured with vials.

Tentative conclusions are as follows:

1. Concentration of 25, 50, and 100 flies per cage--no appreciable difference in mg. of eggs per female with materials tested.
2. Shallow container vs. deep container--pint jar method far superior to cup.
3. Anesthetization with CO₂--no apparent influence on ovipositional response when anesthetized a minimum of 2 hours before exposure to test materials. Since laboratory tests indicate a direct relationship between length of exposure to CO₂ and recovery time it is standard practice to limit CO₂ exposure of the test insects to a maximum of 15 minutes. The only

Cage	Container for sample	Number of flies	Material	mg. of eggs
			CO_2 anesthesia vs. no CO_2	
11	Pint jar	25 CO_2	3a	130
	do.		IRM	130
12	do.	do.	3a	150
	do.		IRM	140
13	do.	25 no CO_2	3a	80
			IRM	200
14	do.	25 no CO_2	3a	220
			IRM	30

1/ Fresh larval medium without larvae or formalin.
 2/ Fresh larval medium without larvae but containing 0.1% formalin
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1. Concentration of 25, 50, and 100 flies per cage--no appreciable difference in mg. of eggs per female with materials tested.
2. Shallow container vs. deep container--pint jar method far superior to cup.
3. Anesthetization with CO_2 --no apparent influence on ovipositional response when anesthetized a minimum of 2 hours before exposure to test materials. Since laboratory tests indicate a direct relationship between length of exposure to CO_2 and recovery time it is standard practice to limit CO_2 exposure of the test insects to a maximum of 15 minutes. The only

change in the testing procedure resulting from this test was the substitution of the pint jars for the paper cups.

In the third test comparisons of the ground heart materials were repeated with 3a again showing the greatest attractancy. Oviposition rates confirmed data in the previous test indicating the superiority of the pint jars.

A further study of the optimum incubation time for sample 3 involved this material incubated for 36, 48, 72, 96, 146, and 192 hours. Two peaks of activity were observed at 36 and 96 hours' incubation with the latter being most attractive. A direct relationship existed between the rise in pH and peak attractiveness.

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